
Quantitative assessment of immune cells in the injured spinal cord tissue by flow cytometry: a novel use for a cell purification method.

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Public Summary:

Scientific Abstract:

Detection of immune cells in the injured central nervous system (CNS) using morphological or histological techniques has not always provided true quantitative analysis of cellular inflammation. Flow cytometry is a quick alternative method to quantify immune cells in the injured brain or spinal cord tissue. Historically, flow cytometry has been used to quantify immune cells collected from blood or dissociated spleen or thymus, and only a few studies have attempted to quantify immune cells in the injured spinal cord by flow cytometry using fresh dissociated cord tissue. However, the dissociated spinal cord tissue is concentrated with myelin debris that can be mistaken for cells and reduce cell count reliability obtained by the flow cytometer. We have advanced a cell preparation method using the OptiPrep gradient system to effectively separate lipid/myelin debris from cells, providing sensitive and reliable quantifications of cellular inflammation in the injured spinal cord by flow cytometry. As described in our recent study (Beck & Nguyen et al., Brain. 2010 Feb; 133 (Pt 2): 433-47), the OptiPrep cell preparation had increased sensitivity to detect cellular inflammation in the injured spinal cord, with counts of specific cell types correlating with injury severity. Critically, novel usage of this method provided the first characterization of acute and chronic cellular inflammation after SCI to include a complete time course for polymorphonuclear leukocytes (PMNs, neutrophils), macrophages/microglia, and T-cells over a period ranging from 2 hours to 180 days post-injury (dpi), identifying a surprising novel second phase of cellular inflammation. Thorough characterization of cellular inflammation using this method may provide a better understanding of neuroinflammation in the injured CNS, and reveal an important multiphasic component of neuroinflammation that may be critical for the design and implementation of rational therapeutic treatment strategies, including both cell-based and pharmacological interventions for SCI.

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